



IFWO

RAW SEQUENCE LISTING

PATENT APPLICATION: US/10/766,993

DATE: 08/26/2004

TIME: 11:37:09

Input Set : A:\-8-1.app

Output Set: N:\CRF4\08262004\J766993.raw

3 <110> APPLICANT: Chang, Chia-Hwa
 4 Liu, Xiaowen
 5 Lewicki, John A.
 6 Xu, Qiang
 7 Osel, Inc.
 9 <120> TITLE OF INVENTION: Surface Expression of Biologically Active Proteins in
 10 Bacteria
 12 <130> FILE REFERENCE: 016976-000810US
 14 <140> CURRENT APPLICATION NUMBER: US 10/766,993
 15 <141> CURRENT FILING DATE: 2004-01-28
 17 <150> PRIOR APPLICATION NUMBER: US 60/443,619
 18 <151> PRIOR FILING DATE: 2003-01-29
 20 <160> NUMBER OF SEQ ID NOS: 89
 22 <170> SOFTWARE: PatentIn Ver. 2.1
 24 <210> SEQ ID NO: 1
 25 <211> LENGTH: 1765
 26 <212> TYPE: PRT
 27 <213> ORGANISM: Lactobacillus jensenii 1153
 29 <220> FEATURE:
 30 <223> OTHER INFORMATION: genomic C14 cell wall anchor sequence
 32 <220> FEATURE:
 33 <221> NAME/KEY: DOMAIN
 34 <222> LOCATION: (1528)..(1765)
 35 <223> OTHER INFORMATION: CWA200 cell wall associated region with anchor
 36 motif
 38 <220> FEATURE:
 39 <221> NAME/KEY: PEPTIDE
 40 <222> LOCATION: (1730)..(1734)
 41 <223> OTHER INFORMATION: anchor motif, sorting signal, cell wall targeting
 42 region
 44 <400> SEQUENCE: 1
 45 Met Asn Asp Ser Ser Ile Gly Thr Ile Asn Ile Thr Asn Asp Ile Thr
 46 1 5 10 15
 48 Ile Thr Gly Lys Val Asn Gly Leu Thr Thr Ser Gly Ile Ser Asp Ile
 49 20 25 30
 51 Asn Lys His Phe Leu Tyr Leu Gln Ser Glu Gly Ser Ala Arg Asp Leu
 52 35 40 45
 54 Thr Ile Asn Gly Asn Gly His Arg Ile Asn Phe Ala Gly Tyr Ser Ile
 55 50 55 60
 57 Ala Leu Gln Asn Lys Asn Tyr Thr Asn Ala Ala Asn Pro Trp Asn Ile
 58 65 70 75 80
 60 Thr Leu Lys Asp Met Thr Ile Glu Gly Ser Lys Tyr Asp Tyr Ser Pro
 61 85 90 95

(Cps. 6)
ENTERED

RAW SEQUENCE LISTING

DATE: 08/26/2004

PATENT APPLICATION: US/10/766,993

TIME: 11:37:09

Input Set : A:\-8-1.app

Output Set: N:\CRF4\08262004\J766993.raw

```

63 Ile Ser Phe Tyr Gly Arg Lys Ser Asn Thr Glu Asn Ser Lys Leu Thr
64           100           105           110
66 Phe Asp Gly Val Thr Ala Asn Leu Asn Asp Arg Pro Leu Val Asp Lys
67           115           120           125
69 Tyr Gly Glu Asn Leu Pro Val His Phe Ala Gly Glu Asn Asn Ile Thr
70       130           135           140
72 Leu Asn Asn Met Ser Ile Gly Tyr Asn Leu Val Thr Gly Lys Thr Val
73 145           150           155           160
75 Lys Phe Asp Ser Gly Asn Thr Thr Phe Asn Val Asp Gly Lys Val Thr
76           165           170           175
78 Gly Asn Ser Ile Asn Pro Asp Asn Trp Val Ile Arg Ser Thr Glu Asn
79           180           185           190
81 Ala Ser Asn Ser Glu Asn Pro Ser Thr Leu Ile Asn Glu Gly Ala Thr
82       195           200           205
84 Val Thr Ile Asn Ala Lys Ser Asp Asp Leu Arg Gly Ile Tyr Ala Gly
85       210           215           220
87 Arg Gln Leu Thr Ala Gly Gln Pro Ile Tyr Gly Val Thr Val Ile Asn
88 225           230           235           240
90 Gly Thr Leu Asn Ala Lys Met Ala Ala Gly His Ser Thr Ala Ile Trp
91           245           250           255
93 Ser His Asp Leu Glu Ile Gly Lys Lys Gly Asn Val Thr Ile His Thr
94           260           265           270
96 Lys Gln Thr Asn Gln Ala Asp Gly Val Glu Asn Gly Thr Ser Asn Ser
97       275           280           285
99 Val Thr Asn Tyr Asn Gly Thr His Tyr Ala Pro Ile Ser Leu Gly Val
100      290           295           300
102 Gly Pro Ile Ser Ser Val Ala Ser Pro Leu Ser Lys Gln Thr Val Ser
103 305           310           315           320
105 Leu Ile Asn Asn Gly Ser Leu Thr Ile Ile Arg Asp Thr Ala Lys Lys
106           325           330           335
108 Thr Leu Val Pro Leu Ile Ser Met Gly Asp Gly Ser Leu Ser Ser Asn
109           340           345           350
111 Thr Thr Leu Lys Phe Ser Val Gly Ala Gly Ala Thr Leu Asp Leu Gln
112           355           360           365
114 Asp Lys Ala Gly Thr Phe Arg Tyr Gly Ile Glu Pro Ser Thr Pro Leu
115       370           375           380
117 Asn Gly Leu Val Thr Leu Trp Gly Thr Ser Gly Thr Asp Leu Leu Glu
118 385           390           395           400
120 Phe Leu Thr Pro Ala Tyr Val Asn Leu Gln Arg Thr Gly Asp Ile Arg
121           405           410           415
123 Gly Thr Leu Ile Arg Met Glu Gly Val Tyr Asn Ser Thr Thr Val Asn
124           420           425           430
126 Gly Pro Thr Pro Val Ala Gln Trp Asp Gln Gly Asn Lys Thr Thr Thr
127       435           440           445
129 Pro Asn Asp Val Trp Tyr Val Arg Tyr Leu Ile Ser Ala Asn Gln Trp
130       450           455           460
132 Gly Asn Asn Ser Gly Gln Phe Met Gly Lys Asp Gln His Pro Asn Thr
133 465           470           475           480
135 Val Val Ala Lys Lys Gly Val Asp Thr Leu Tyr Asn Ser Asn Ala Thr

```

RAW SEQUENCE LISTING

DATE: 08/26/2004

PATENT APPLICATION: US/10/766,993

TIME: 11:37:09

Input Set : A:\-8-1.app

Output Set: N:\CRF4\08262004\J766993.raw

136				485				490				495		
138	Val	Leu	Met	Ser	Lys	Asn	Gln	Gly	Ala	Asp	Lys	Tyr	Glu	Asn
139				500					505				510	
141	Met	Pro	Thr	Glu	Val	Gln	Gln	Ala	Leu	His	Leu	Asn	Ser	Phe
142				515				520					525	
144	Asn	Phe	Asn	Phe	Trp	Arg	Pro	Gln	Arg	Met	Ala	Met	Gly	Ser
145				530				535					540	
147	Asn	Asp	Asn	Pro	Asp	Val	Lys	Ile	Asp	Asp	Phe	Asp	Lys	Tyr
148				545				550					555	
150	Glu	Ala	Gln	Thr	Ile	Asp	Gly	Thr	Thr	Arg	Gln	Thr	Leu	Ser
151					565					570			575	
153	Asp	Ala	Asn	Lys	Gly	Leu	Lys	Asp	Leu	Ile	Gly	Pro	Asp	Glu
154				580					585				590	
156	Ile	Thr	Asp	Phe	Lys	Asp	Ile	Val	Lys	His	Val	Thr	Trp	Tyr
157				595				600					605	
159	Ala	Thr	Asp	Lys	Asp	Glu	Trp	Asn	Lys	Ile	Met	Ile	Gln	Pro
160				610				615					620	
162	Ser	Lys	Asp	Pro	Ser	Ala	Arg	Val	Pro	Tyr	Pro	Glu	Pro	Gln
163				625				630					635	
165	Thr	Gly	Asn	Leu	Lys	Thr	Thr	Asp	Gly	Phe	Ala	Trp	Ala	Lys
166					645					650			655	
168	Tyr	Ala	Asp	Gly	Ser	Val	Asp	Phe	Val	Lys	Ile	Pro	Leu	Lys
169				660					665				670	
171	Glu	Lys	Lys	Tyr	Ser	Glu	Glu	Leu	Thr	Pro	Ser	Tyr	Pro	Gly
172				675				680					685	
174	Val	Glu	Gln	Gly	Lys	Ser	Asp	Ser	Val	Asp	Pro	Ser	Phe	Lys
175				690				695					700	
177	Asn	Asp	Lys	Ala	Ala	Asp	Ala	Pro	Ala	Gly	Thr	Lys	Tyr	Thr
178				705				710					715	
180	Glu	Asn	Thr	Pro	Asp	Trp	Ile	Lys	Val	Asp	Pro	Asp	Thr	Gly
181					725					730			735	
183	Thr	Val	Ser	Pro	Thr	Asp	Asp	Thr	Ser	Val	Gly	Ser	His	Asp
184				740					745				750	
186	Val	Thr	Val	Thr	Tyr	Pro	Asp	Ser	Ser	Thr	Asp	Gln	Leu	Thr
187				755				760					765	
189	Val	Thr	Val	Thr	Glu	Lys	Ser	Asn	Leu	Ala	Glu	Lys	Tyr	Pro
190				770				775					780	
192	Tyr	Asp	Lys	Leu	Asn	Val	Glu	Lys	Pro	Ser	Gly	Asp	Thr	Pro
193				785				790					795	
195	Gly	Ala	Val	Asp	Pro	Lys	Ala	Ala	Ala	Asp	Met	Pro	Glu	Gly
196					805					810			815	
198	Thr	Gly	Tyr	Glu	Lys	Gly	Asp	Phe	Asp	Ala	Pro	Ala	Gly	Val
199				820					825				830	
201	Asp	Val	Asn	His	Asp	Thr	Gly	Lys	Val	Thr	Ala	Ser	Val	Gly
202				835				840					845	
204	Ala	Thr	Leu	Gly	Ser	Phe	Glu	Val	Pro	Val	Lys	Val	Thr	Tyr
205				850				855					860	
207	Gly	Thr	Tyr	Ala	Glu	Val	Lys	Val	Pro	Val	Ser	Ile	Thr	Gly
208				865				870					875	
													880	

RAW SEQUENCE LISTING

DATE: 08/26/2004

PATENT APPLICATION: US/10/766,993

TIME: 11:37:09

Input Set : A:\-8-1.app

Output Set: N:\CRF4\08262004\J766993.raw

```

210 Val Asp Pro Gly Ser Gly Asp Val Val Tyr Tyr Gly Asp Gln Ser Met
211      885      890      895
213 Val Val Phe Asn Gly Asn Leu Thr Thr Val His Lys Thr Thr Asp Ser
214      900      905      910
216 His Glu Leu Ser Ala Lys Asp Ser Ala Phe Gln Thr Ile Thr Tyr Tyr
217      915      920      925
219 Ser Asp Trp Asn Lys Lys Gly Asn Ile Val Ser Asp Tyr Asn Lys His
220      930      935      940
222 Val Ile Tyr Lys Leu Ser Ala Asp Gly Thr Lys Tyr Val Asn Glu Ala
223 945      950      955      960
225 Asp Ala Thr Asp Ser Phe Asp Ala Ser Ala Ile Ser Phe Asn Trp Gln
226      965      970      975
228 Lys Gly Tyr Glu Val Asn Thr Gly Val Asp Asn Phe Ser Asn Gly Ser
229      980      985      990
231 Ala Asp Thr Leu Tyr Gln Leu Glu Lys Gly Ala Val Asn Ser Glu Glu
232      995      1000      1005
234 Gln Thr Asp Ala Asn Asp Pro Ser Gly Leu Ala Gly Asn Ser Lys Tyr
235      1010      1015      1020
237 Arg Tyr Asp Phe Ser Ile Ser Asp Thr Asn Val Leu Gln Lys Leu Gly
238 1025      1030      1035      1040
240 Leu Ser Pro Ala Gly Tyr Asn Ala Trp Ala Asn Val Tyr Tyr Asn Phe
241      1045      1050      1055
243 Leu Gly Ala Thr Gly Lys Ile Asn Ile Pro Val Asn Tyr Gly Ser Glu
244      1060      1065      1070
246 Val Ser Thr Asp Glu Ala Gly Ile Lys Asn Tyr Leu Ala Thr Asn Ser
247      1075      1080      1085
249 Ile Ser Gly Lys Thr Phe Val Asn Gly Asn Pro Thr Gly Ile Lys Trp
250      1090      1095      1100
252 Ala Glu Asn Gly Met Pro Gly Lys Asp Gly Lys Phe Ala Ala Ser Asn
253 1105      1110      1115      1120
255 Met Thr Gly Ile Val Glu Phe Thr Phe Asp Asn Gly Thr Lys Leu Asn
256      1125      1130      1135
258 Val Gln Val Thr Phe Lys Thr Gly Ser His Val Ser Thr Ser Gly Ser
259      1140      1145      1150
261 Lys Val Asn Asp Asp Thr Asn Leu Tyr Val Glu Arg Thr Ile Glu Tyr
262      1155      1160      1165
264 Asp Val Thr Gly Thr Gly His Ser Pro Ile Asn Ser Val Thr Gln Lys
265      1170      1175      1180
267 Val His Tyr Val Arg Asp Gly Tyr His Lys Ile Asn Ala Asp Gly Thr
268 1185      1190      1195      1200
270 Asp Ala Gly Glu Ile Ile Trp Asn Glu Trp Lys Leu Ala Asp Gly Gln
271      1205      1210      1215
273 Thr Ala Glu Phe Pro Glu Tyr Ser Val Asp Gln Ile Thr Gly Tyr Asp
274      1220      1225      1230
276 Ala Tyr Ile Asn Gly Ala Lys Ala Thr Gln Val Asp Ala Ala Lys Val
277      1235      1240      1245
279 Ala Glu Thr Asn Gly Thr Pro Gln Asn Gly Gln Asn Ile Thr Val Thr
280      1250      1255      1260
282 Tyr Lys Lys Gln Asn Ser Thr Pro Val Pro Tyr Lys Pro Gly Lys Asp

```

RAW SEQUENCE LISTING

DATE: 08/26/2004

PATENT APPLICATION: US/10/766,993

TIME: 11:37:09

Input Set : A:\-8-1.app

Output Set: N:\CRF4\08262004\J766993.raw

```

283 1265          1270          1275          1280
285 Gly Val Asn Asp Ala Ile Asn Arg Tyr Val Thr Arg Thr Ile Ile Val
286          1285          1290          1295
288 Lys Glu Pro Gly Lys Glu Pro Gln Thr Ile Thr Gln Thr Val His Phe
289          1300          1305          1310
291 Thr Asn Glu Asp Lys Asp Gly Asn Ser Gly Tyr Lys Asp Pro Val Thr
292          1315          1320          1325
294 Gly Glu Ile Lys Tyr Asn Thr Asp Trp His Val Ala Ser Asp Leu Asn
295          1330          1335          1340
297 Ala Lys Thr Gly Ser Trp Glu Glu Tyr Thr Ala Pro Ser Val Thr Gly
298 1345          1350          1355          1360
300 Tyr Thr Pro Ser Gln Ala Lys Val Glu Ala Lys Thr Val Thr Ala Glu
301          1365          1370          1375
303 Thr Glu Ala Ala Ser Val Thr Ile Ser Tyr Thr Lys Asn Ala Asp Ile
304          1380          1385          1390
306 Pro Val Pro Tyr Lys Pro Gly Lys Asp Gly Val Asn Asp Ala Ile Asn
307          1395          1400          1405
309 Arg Tyr Val Thr Arg Thr Ile Ile Val Lys Glu Pro Gly Lys Glu Pro
310          1410          1415          1420
312 Gln Thr Ile Thr Gln Thr Val His Phe Thr Asn Glu Asp Lys Asp Gly
313 1425          1430          1435          1440
315 Asn Ser Gly Tyr Lys Asp Pro Val Thr Gly Glu Ile Lys Tyr Asn Thr
316          1445          1450          1455
318 Asp Trp His Val Ala Ser Asp Leu Asn Ala Lys Thr Gly Ser Trp Glu
319          1460          1465          1470
321 Glu Tyr Thr Ala Pro Ser Val Thr Gly Tyr Thr Pro Ser Gln Ala Lys
322          1475          1480          1485
324 Val Glu Ala Lys Thr Val Thr Ala Glu Thr Glu Ala Ala Ser Val Thr
325          1490          1495          1500
327 Ile Ser Tyr Thr Lys Asn Ala Asp Ile Pro Val Pro Phe Asp Pro Ser
328 1505          1510          1515          1520
330 Asn Lys Asp Met Tyr Arg Glu Val Thr Arg Thr Ile Asn Val Val Asp
331          1525          1530          1535
333 Pro Ile Thr Gly Lys Ile Ser Thr Ser Val Gln Thr Ala Lys Phe Thr
334          1540          1545          1550
336 Arg Glu Asp Lys Asn Ser Asn Ala Gly Tyr Thr Asp Pro Val Thr Gly
337          1555          1560          1565
339 Lys Thr Thr Met Asn Pro Trp Thr Pro Ala Lys Gln Gly Leu Arg Ala
340          1570          1575          1580
342 Val Asn Val Glu Gln Ile Lys Gly Tyr Val Ala Lys Val Asp Gly Asn
343 1585          1590          1595          1600
345 Val Asp Ala Val Val Val Thr Pro Asp Ser Ala Asn Met Val Val Thr
346          1605          1610          1615
348 Ile Thr Tyr Gln Ala Asn Lys Pro Glu Gly Gln Asn Ile Thr Val Lys
349          1620          1625          1630
351 Lys Asp Thr Val Pro Asp Pro Ala Asp Gly Ile Lys Asn Lys Asp Asp
352          1635          1640          1645
354 Leu Pro Asp Gly Thr Lys Tyr Thr Trp Lys Glu Val Pro Asp Val Asn
355          1650          1655          1660

```

RAW SEQUENCE LISTING ERROR SUMMARY
PATENT APPLICATION: US/10/766,993

DATE: 08/26/2004
TIME: 11:37:10

Input Set : A:\-8-1.app
Output Set: N:\CRF4\08262004\J766993.raw

Please Note:

Use of n and/or Xaa have been detected in the Sequence Listing. Please review the Sequence Listing to ensure that a corresponding explanation is presented in the <220> to <223> fields of each sequence which presents at least one n or Xaa.

Seq#:9; Xaa Pos. 3
Seq#:29; Xaa Pos. 3
Seq#:30; Xaa Pos. 3
Seq#:31; Xaa Pos. 3,6

VERIFICATION SUMMARY

PATENT APPLICATION: US/10/766,993

DATE: 08/26/2004

TIME: 11:37:10

Input Set : A:\-8-1.app

Output Set: N:\CRF4\08262004\J766993.raw

L:1221 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:9 after pos.:0
L:1527 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:29 after pos.:0
L:1546 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:30 after pos.:0
L:1566 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:31 after pos.:0